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Immunohistochemical Analysis of the Poorly Differentiated Stomach Adenocarcinoma With Medullary Growth Pattern

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Poorly differentiated gastric adenocarcinoma with medullary features (poor medullary) is distinguished by a propensity for hepatic metastasis. To classify it antigenically, we compared it to poorly differentiated adenocarcinoma with scirrhous growth pattern (poor scirrhous), well and moderately differentiated adenocarcinoma (differentiated adenocarcinoma), and normal gastric mucosa (foveolar and deep epithelium) using immunohistochemistry with antibodies against CEA, AFP, NSE, and Lewis-type antigens. Lewis^a antigen was significantly associated with differentiated adenocarcinoma and foveolar epithelium, although Lewis^x antigen was significantly expressed in poor medullary, poor scirrhous, and deep gland epithelium. From the viewpoint of expression of Lewis^a, there was no significant differentiation between poor medullary and differentiated adenocarcinoma, but it was definite between poor scirrhous and differentiated adenocarcinoma. Therefore, we conclude that in antigenic expression, poor medullary carcinoma is allied with differentiated adenocarcinoma rather than poorly differentiated scirrhous carcinoma.

KEY WORDS: **gastric cancer, poorly differentiated adenocarcinoma, medullary growth pattern, blood-related Lewis antigens**

INTRODUCTION

Gastric cancer that demonstrates ductal hypoplasia is classified into poorly differentiated adenocarcinoma and signet ring cell carcinoma according to the General Rules for Gastric Cancer Study [1]. Most of them infiltrate scirrhous fibrous stroma, but poorly differentiated adenocarcinoma is thought to have different clinical and pathological characteristics [2]. For example, their metastasis is said to take mainly the forms of peritoneal dissemination or lymph node metastasis and rarely to take the form of hematogenous metastasis, including the liver, whose forms are regarded as quite different from that of well or moderately differentiated adenocarcinoma of the stomach [3,4]. However, even in poorly differentiated adenocarcinoma, which only rarely accompanies duct formation, poor medullary that shows medullary or solid growth pattern often metastasizes to the liver [2]. It shows a characteristic metastatic behavior not in accordance with other types of poorly differentiated adenocarcinoma. Thus it seemed worthwhile to elucidate the pathological characteristics of poor medullary, especially its immunohistological difference from that of poor scirrhous. For this reason, we conducted an immunohistological study of poor medullary on each of the following antigens: Lewis^a (Le^a), Lewis^b (Le^b), Lewis^x (Le^x), and Lewis^y (Le^y). The aim was to clarify the characteristic patterns of expression of blood group-related antigens that change their modes of expression as the differentiation and canceration proceed [5,6] in digestive tract cancers.

We also studied the expression of neurospecific enolase (NSE) in addition to CEA and AFP, because it had been

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TABLE I. Expression of Blood Group-Related Antigens, CEA, AFP, and NSE in 28 Cases of Poorly Differentiated Adenocarcinoma With Medullary Growth Pattern of the Stomach

Case					Antigens						
no.	Age	Sex	Site[a]	Stage	Lewis[a]	Lewis[b]	Lewis[x]	Lewis[y]	CEA[b]	AFP[c]	NSE[d]
1	28	F	M	I_b	−	+	−	−	−	−	+
2	67	M	M	III_b	−	+	+	+	−	−	+
3	49	F	C	III_b	+	−	+	−	+	−	−
4	53	M	C	III_a	−	−	+	−	−	−	−
5	77	M	C	II	−	−	+	+	−	−	−
6	63	F	M	III_a	−	−	+	−	−	−	+
7	56	M	M	I_a	−	−	+	+	−	−	−
8	73	M	A	III_b	+	+	+	+	−	−	−
9	52	M	C	IV_a	−	+	+	−	−	−	−
10	65	M	A	II	+	+	−	+	−	−	−
11	67	M	M	IV_a	−	−	+	−	−	−	−
12	44	F	M	III_a	−	+	+	−	+	−	−
13	73	F	A	I_b	−	−	−	−	−	+	−
14	57	M	M	II	−	−	−	−	−	−	+
15	67	M	A	II	−	+	+	+	−	−	−
16	60	M	M	III_b	−	−	−	+	−	−	+
17	58	M	A	III_a	−	−	−	−	−	−	+
18	74	M	A	I_a	−	−	+	−	−	−	−
19	66	M	C	I_b	−	−	−	−	−	−	+
20	71	M	C	I_a	−	−	+	−	−	−	−
21	71	M	C	III_b	+	+	+	+	+	−	−
22	42	M	C	III_b	+	+	+	+	+	−	−
23	51	M	C	IV_b	−	−	+	−	−	−	−
24	63	F	M	III_a	−	−	+	+	+	−	−
25	52	F	M	I_a	+	+	+	−	+	−	−
26	48	M	M	II	+	−	+	−	+	−	−
27	51	M	C	II	−	−	+	−	−	−	+
28	91	F	A	II	−	−	+	+	−	−	−

[a]M: midbody of the stomach, C: cardia of the stomach, A: antrum of the stomach.
[b]Carcinoembryonic antigen.
[c]α-fetoprotein.
[d]Neuro-specific enolase.

reported that poor medullary included cells that had neurosecretory granulation [7]. As a reference for comparison, we studied the expression of these blood group-associated antigens through immunohistological staining of differentiated adenocarcinoma and poor scirrhous.

MATERIALS AND METHODS

Among gastric cancer cases undergoing curative resection at the Department of Surgery II, School of Medicine, Nagoya University, from January 1984 to December 1987, 28 patients who were histopathologically diagnosed as poor medullary were the subjects of this study. In order to study the correlation between the pathological characteristics and the antigen distribution of poor medullary, we also studied the distribution of antigens in 19 cases of poor scirrhous and 21 cases of differentiated adenocarcinoma. Expression of blood group-related antigens was also studied on normal mucosa adjacent to poor medullary. Of the 28 patients with poor medullary, 20 were male and 8 were female. Ages of the patients ranged from 28 to 91 years (average 58.4 years). Location of the lesion was antrum of stomach in 7 cases, body of stomach 11 and cardia 10, respectively. Staging distribution is shown in Table I. No cases had liver metastasis or peritoneal dissemination at the time of operation (Table I).

Immunohistological Staining

The immunoperoxidase staining method (ABC method, Vector Laboratory, Burlingame, CA) was employed as follows. Thin serial sections 4 μm thick, were cut from paraffin-embedded tissue blocks, deparaffinized, and incubated in 100% methanol with 0.3% hydrogen peroxide added to remove endogenous peroxidase activity, and subjected to diluted normal serum dripped on them to prevent nonspecific binding. The sections were then left to react with the primary antibody at room temperature for 1 hour, washed, and left to react with the secondary antibody of biotinylated antimouse IgG at room temperature for 30 min. The sections were washed in PBS, then incubated in avidin DH and biotinylated

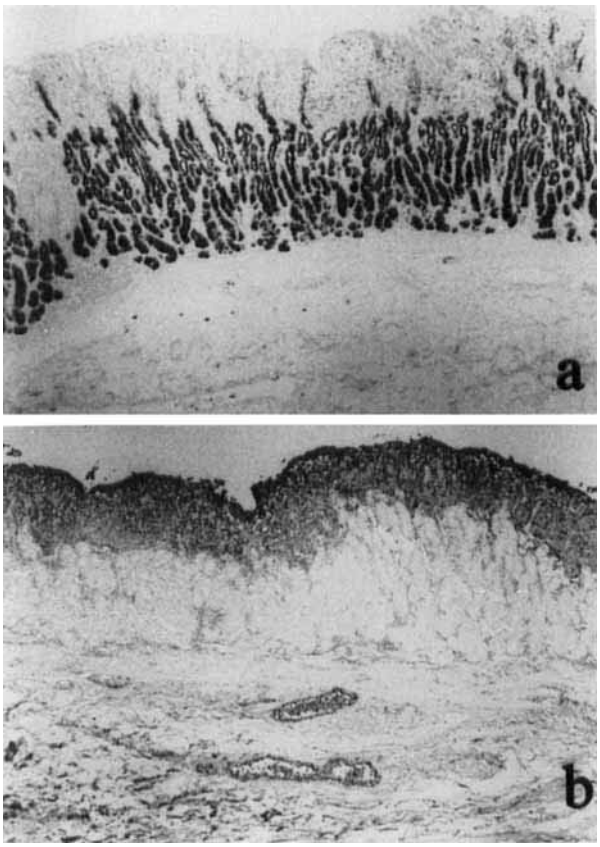

Fig. 1. Localization of Lewis blood group associated antigens in normal mucosa and gastric cancer. (a) Gastric normal mucosa was uniformly stained by Le^x. (b) Gastric cancer was stained as uniformly as gastric normal mucosa be Le^b.

horseradish peroxidase H at room temperature for 45 min. Next, sections were washed and immersed in peroxidase substrate for 5 min for staining, followed by nuclear staining in 1% hematoxylin-eosin solution. The sections were then dewatered and mounted. Monoclonal antibodies used in the experiment were: anti-Le^a antibody T-174 [8], anti-Le^b antibody T-218 [9], anti-Le^x antibody P-12 [10], anti-Le^y F-3 [11], and anti-CEA antibody 102 [12]. For AFP and NSE, polyclonal antibodies, anti-AFP antibody (PG-012, supplied by NBT, Tokyo) and anti-NSE antibody (P-036, supplied by IRO, Umea, Sweden), both polyclonal antibodies were used. In the determination of antigen distribution samples in which entire tumor or normal mucosa were stained uniformly were regarded to be positive, and other samples were regarded as negative (Fig. 1).

Statistical Evaluation

The Chi-square test was employed for the statistical evaluation. A significant difference was acknowledged only when the P value was <0.05.

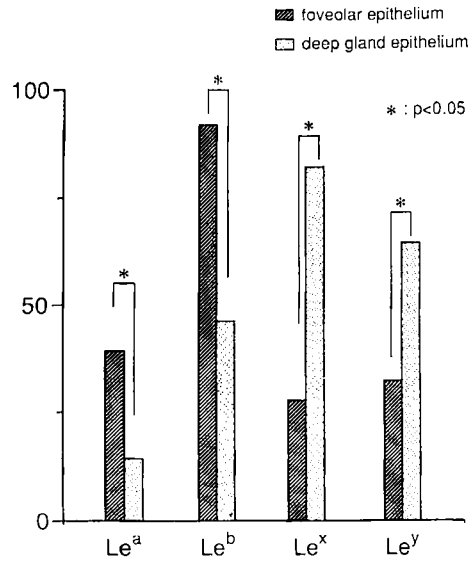

Fig. 2. Frequency of expression of blood group associated antigens in gastric normal mucosa.

RESULTS

Expression of Blood Group-Associated Antigen in Normal Gastric Mucosa

We studied the frequency of expression of the blood group-associated antigens in normal gastric mucosa (Fig. 2). Of the blood group-associated antigens, Le^a and Le^b, which are type 1 antigens, were expressed more strongly in foveolar epithelium than in deep gland epithelium. There was a significant difference with respect to expression of Le^a and Le^b ($P < 0.05$). Conversely, Le^x and Le^y, the type 2 antigens, were expressed more strongly in deep gland than in gastric foveolar epithelium. There was also a significant difference in connection with distribution of Le^x and Le^y ($P < 0.05$). In spite of the same normal gastric mucosa, foveolar epithelium and deep gland epithelium had considerably different characteristics from the viewpoint of expression of blood group-associated antigens. The expression of Le^a and Le^b was mainly in the foveolar epithelium, and Le^x and Le^y were in the deep gastric gland (Fig. 3).

Expression of Blood Group-Associated Antigens, CEA, AFP, and NSE, in Poor Medullary

An immunohistological study was conducted on poor medullary. As shown in Table I, the expression of Le^a, Le^b, Le^x, Le^y, CEA, AFP, and NSE was confirmed in 9, 10, 21, 12, 7, 1, and 8 cases, respectively. Expression of Le^x is recognized more than type 1 blood group antigens of Le^a and Le^b ($P < 0.01$). Frequency of Le^y was similar to those of Le^a and Le^b. Therefore, Le^x could be said to be the major antigen which expresses the basic character of poor medullary. Positive cases of Le^a, Le^b, Le^x, and Le^y are demonstrated in Figure 4. As indicated in Table

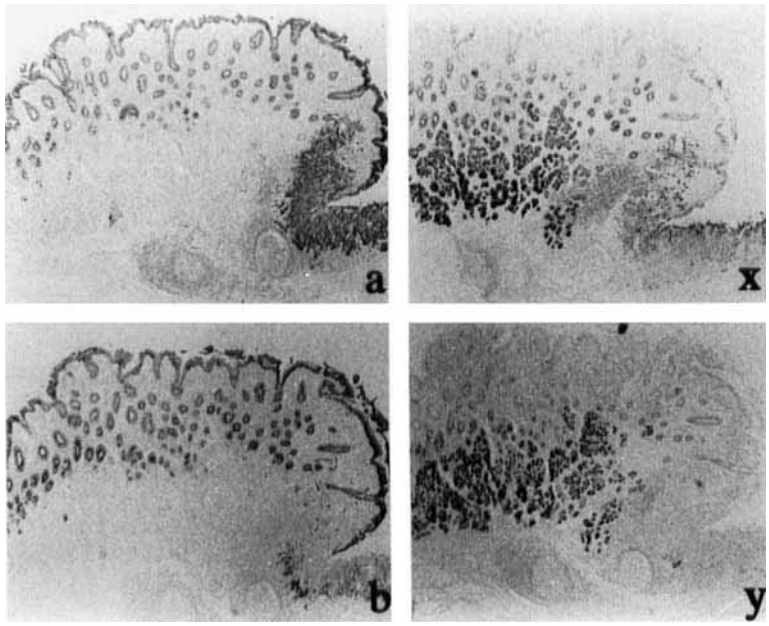

Fig. 3. Localization of Lewis blood group-related antigens in gastric normal mucosa. Le^a and Le^b are stained mainly in foveolar epithelium. Conversely, Le^x and Le^y are stained mainly in deep gland. **a**:Le^a, **b**:Le^b, **x**:Le^x, **y**:Le^y.

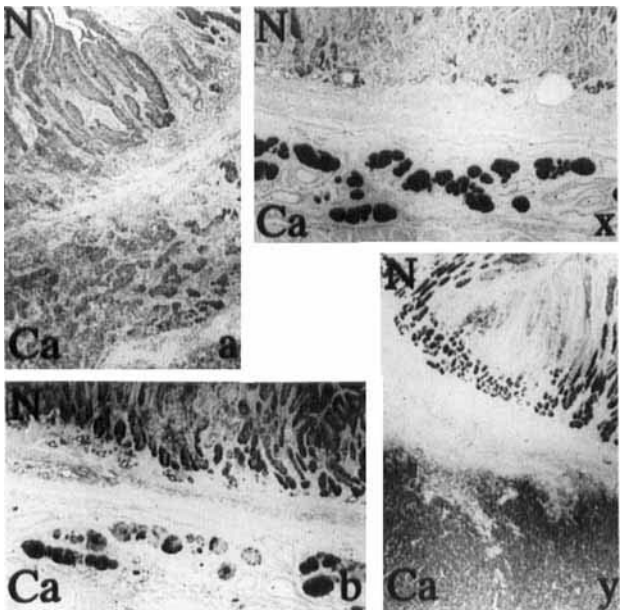

Fig. 4. These are the positive cases of Le^a, Le^b, Le^x, and Le^y antigens in gastric cancer. Ca: cancer, N: normal mucosa, **a**:Le^a, **b**:Le^b, **x**:Le^x, **y**:Le^y.

I and Figure 5, CEA antigen was expressed in seven cases (25%), but it was never distributed in normal gastric mucosa. Of these seven cases, high serum CEA (>5.0 ng/ml) was observed in only two cases, whereas AFP was positive in only one case (3.6%) as shown in Figure 5. This case also showed an unusually high serum AFP of 7,250 ng/ml. In addition, there were two cases with high serum AFP (65.7 ng/ml and 131 ng/ml, respectively), but these cases demonstrated no AFP expression in the cancer lesion. Expression of NSE antigen was confirmed in the cancer tissues of eight cases (28.6%) (Table I, Fig. 5). Cases with positive expression of CEA, AFP, and NSE were distributed as shown in Table I. No tumor expressed more than one of the antigens other than Lewis. It was also confirmed that all of CEA positive cases are included in type 1 blood group antigen-positive cases. However, all but two AFP and NSE positive cases had no expression of type 1 blood group antigens. From the distribution of CEA, AFP, and NSE antigens, poor medullary could be classified into several groups.

Expression of Blood Group-Associated Antigens in Poor Medullary, Poor Scirrhous, and Differentiated Adenocarcinoma

First, we examined the frequency of blood group antigens in poorly differentiated adenocarcinoma (poor medullary and poor scirrhous, 47 cases) and differentiated adenocarcinoma. As indicated in Figure 6, Le^a was expressed more often in differentiated adenocarcinoma ($P < 0.03$). Conversely, Le^x was distributed more frequently in poorly differentiated adenocarcinoma ($P < 0.01$), whereas Le^b and Le^y were recognized equally in both poorly differentiated adenocarcinoma and differentiated adenocarcinoma. These results indicate that Le^a and Le^x are the key antigens of differentiated adenocarcinoma and poorly differentiated adenocarcinoma respectively. Then, we examined the frequency of these antigens

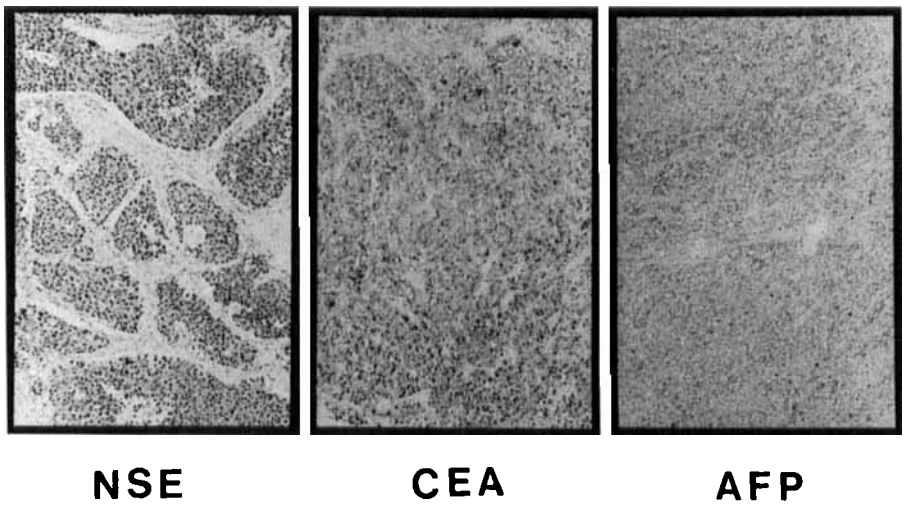

Fig. 5. These are the positive cases of NSE, CEA, and AFP, respectively. They also stained as uniformly as Lewis antigens. NSE: neuro specific enolase, CEA: carcinoembrionic antigen, AFP: a-fetoprotein.

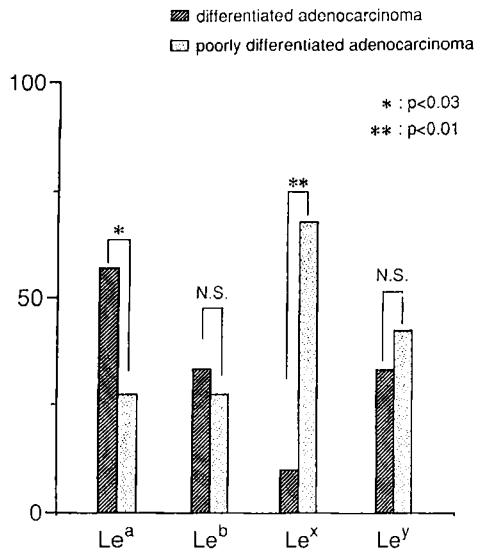

Fig. 6. Expression of the blood group-related antigens in poorly differentiated adenocarcinoma and differentiated adenocarcinoma.

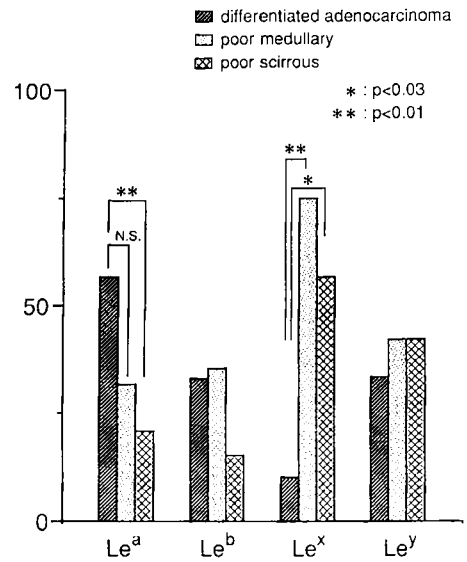

Fig. 7. Frequency of expression of blood group-related antigens in poor medullary, poor scirrhous, and differentiated adenocarcinoma.

among poor medullary, poor scirrhous, and differentiated adenocarcinoma. As shown in Figure 7, Le^x was distributed more in poor medullary and poor scirrhous than in differentiated adenocarcinoma ($P < 0.01$). The expression of Le^a was confirmed to occur more in differentiated adenocarcinoma than in poor scirrhous ($P < 0.03$), but there was no significant difference between poor medullary and differentiated adenocarcinoma. Le^b and Le^y were confirmed similarly among these three groups. From these results, it is suggested that poor medullary basically has the characteristics of poorly differentiated adenocarcinoma, but it shows more characteristics similar to differentiated adenocarcinoma than poor scirrhous.

DISCUSSION

Blood group-associated antigens exist not only in red blood cells but in various kinds of other cells, tissues, and organs of the whole body. It has become clear that these antigens undergo significant changes in terms of both quantity and quality as the cells undergo differentiation or carcinogenesis [5,6]. The blood group-associated antigens can be classified into Le^a and Le^b, which belong to the type 1 antigen, and Le^x and Le^y, which belong to the type 2 antigen. In the present study, in order to elucidate the pathological nature of poor medullary, which shows a peculiar mode of metastasis different from other types of poorly

differentiated adenocarcinomas, we investigated the expression of CEA, AFP, and NSE, the so-called digestive tract epithelium-related antigens, through comparison with normal gastric mucosa, poor scirrhous, and differentiated adenocarcinoma.

Various reports have been published on the expression of blood group-associated antigens in normal gastric mucosa. Ernst et al. [13] reported Le^a in foveolar epithelium but Le^b in deep gland epithelium. Cordon-Cardo et al. [14] found no Le^a in foveolar epithelium but Le^b in both foveolar epithelium and deep gland epithelium. Sakamoto et al. [15] reported that Le^a and Le^b expressed mainly in foveolar epithelium and that Le^x and Le^y expressed mainly in the deep gastric gland epithelium. In the present study, type 1 antigens Le^a and Le^b were expressed predominantly in foveolar epithelium among normal mucosa tissues, and type 2 antigens Le^x and Le^y were expressed predominantly in the deep gastric gland as shown in Figures 2 and 3. Thus even in normal gastric epithelium, expression of blood group-associated antigens is quite different between the foveolar epithelium and the deep gland epithelium and also suggests a difference between these and normal gastric mucosa.

In poor medullary or poor scirrhous, type 2 antigens Le^x and Le^y were expressed at a higher rate, whereas in differentiated adenocarcinoma, type 1 antigens Le^a and Le^b were expressed predominantly (Figs. 6 and 7). This suggests that in terms of antigen expression, differentiated adenocarcinoma is closely associated with the superficial foveolar epithelium and poorly differentiated adenocarcinoma is closely associated with the deep gland epithelium. Further examination of the poor medullary revealed that type 1 antigen Le^a had an intermediate rate of expression between those of differentiated adenocarcinoma and poor scirrhous and that of Le^b a rate of expression nearly equal to that of differentiated adenocarcinoma.

In conclusion, poor medullary resembles differentiated adenocarcinoma more than poor scirrhous, although its pathological nature is basically that of poorly differentiated adenocarcinoma. Although metastasis to the liver reportedly is frequently observed in cases of differentiated adenocarcinoma, the fact that the mode of antigen expression of poor medullary is similar to that of differentiated adenocarcinoma might explain why poor medullary shows more frequent liver metastasis than poor scirrhous [2]. In poor medullary cases, a higher rate of Le^x expression was evident than in poor scirrhous or differentiated adenocarcinoma. Indeed, Le^x may not be used as a tumor marker, because it is expressed also in normal gastric epithelium as well as in cancer tissues. However, it may be a useful indicator in monitoring, because CSLEX (sialylated Le^x) is expressed similarly to Le^x and is rarely found in normal mucosa. As shown in Table I, no case showed positive expression to double antigens. These results suggest that poor medullary may be divided into subgroups based on the expression of NSE, AFP, and CEA and that each has specific clinicopathological features depending on the subgroup. Most (6 cases) of the CEA-positive cases showed expression of type 1 antigens (especially Le^a). CEA-positive cases particularly, among the poor medullary, seemed to have characteristics similar to those of differentiated adenocarcinoma.

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